REMARKS

The amended claims are based on original claims 16, 17, 18, 20 and 23 as set forth in the National Phase Entry filed on September 4, 2001. The claims have been recast to describe methods for producing plant cells that accumulate carotenoids, which cells are normally carotenoid-free, via transformation of said cells with particular expression cassettes. Also claimed are transformed plant cells obtainable by such methods. Applicants had elected to prosecute the claims of Group I in a Response to Restriction Requirement that was mailed on May 26, 2004. Group I included original claims 16, 17, 18, 20 and 23.

This invention is based on the demonstration that it is possible to initiate and accumulate carotenoid production in cells that are normally carotenoid-free. The person skilled in the art will recognise that it is no simple task to recreate a functional biochemical pathway in a cell that is normally absent the pathway. There is, understandably, much technical uncertainty in initiating and maintaining a pathway within a cell that does not normally comprise the pathway. All appropriate elements for the pathway need to be present, in the appropriate quantities and at the appropriate times. The cells own internal mechanisms need to be considered as well. Will any of the products of the pathway have negative effects on the cell either directly or indirectly? Notwithstanding this uncertainty, we have demonstrated that it is possible to produce plant cells that can accumulate carotenoids, which cells are normally carotenoid-free, via the methods as presently claimed.

We believe that the amended claims are supported by on the application as filed and do not add any new matter. There follows a description of the basis with reference to the International Application as published (WO00/53768) and the claims presently on file.

Claim 1 – Based on original claims 16, 17 and 18. The term "accumulate carotenoids" is present on page 7 line 23. "[N]ormally carotenoid free" is described in original claim 18, page 5 lines 1 to 5 and page 9 lines 18 to 24. Transforming "plant material" is described on page 19 line

31. The expression cassette being a phytoene synthase and phytoene desaturase from a plant is described in original claims 4, 5 and 11, page 7 lines 26 to 32 and Figure 4 "Plasmid A".

Claims 2 and 3 – Basis on page 3 line 26 and Figure 4 "Plasmid A".

Claim 4 – Basis in original claim 6 and page 11 lines 5 to 10.

Claim 5 – Basis on page 11 lines 29-30, page 12 lines 1-6 and "Plasmid A".

Claim 6 – Based on "Plasmid A".

Claim 7 – Based on original claim 5.

Claim 8 – Based on "Plasmid A" and page 22 lines 6-7.

Claim 9 – Based on original claim 3 and page 22 lines 20-25.

Claim 10 – Based on original claim 10 and page 24 lines 4-13.

Claim 11 – Based on original claim 12 and page 23 line 26 to page 24 line 13.

Claim 12 – Based on page 24 line 20 to page 25 line 13.

Claim 13 – Based on original claim 13 and page 24 lines 11-13.

Claim 14 – Based on original claim 15, page 5 line 3, page 10 line 6, page 22 line 20, page 23 line 26 to page 24 line 13 and page 27 line 16.

There follows comments in response to the objections raised by the Examiner in the order that they appear in the Official Action.

I. Drawings

Please note that Applicants have submitted the replacement drawings.

II. Rejections under 35 U.S.C. § 112

The amended claims now recite a method for producing plant cells that accumulate carotenoids which cells are normally carotenoid-free. The claimed method involves the use of an expression cassette capable of directing expression in the plant cell of a phytoene synthase derived from a plant and an expression cassette capable of directing production in said cell of a phytoene desaturase derived from bacteria.

The examples and in particular "Plasmid A" specifically describe the use of a phytoene synthase gene from daffodil (*Narcissus pseudonarcissus*) and the phytoene desaturase gene from bacteria (*Erwinia uredovora*). In addition, the application as filed (on page 3 line 24-32) describes that such carotenoid biosynthesis genes can be cloned from a variety of organisms ranging from bacteria to plants. This description provides examples of such bacteria and plants. Furthermore, page 8 lines 6-10 discloses that a large, still increasing number of genes coding for phytoene synthase and desaturase have been isolated and are accessible from the databases. Applicants therefore believe that the Written Description and enablement requirements in respect of the subject matter of the amended claims are fulfilled.

The Examiner's comments in respect of fungal enzymes are moot since the amended claims make no reference to fungal enzymes.

The Examiner has also stated that the state of the art for isolating and using phytoene synthase encoding polynucleotides from plants is unpredictable because so few of these genes have been isolated and studied. Applicants respectfully disagree. As mentioned above, the application as filed indicates that an increasing number of such genes coding for phytoene synthase are accessible from the public databases. It is clear from the application as filed that the phytoene synthase gene when used in accordance with the present invention performs a particular and specific role. This role, referred to on page 2 line 15 as the "first carotenoid-specific reaction" is the conversion of geranylgeranyl diphosphate (GGPP) to Phytoene and is catalytically achieved via phytoene synthase. Thus, the person skilled in the art would

understand that a phytoene synthase when used in the context of this invention is an enzyme that is capable of catalysing the metabolism of GGPP to phytoene.

In addition to this, Applicants have demonstrated that particular sequences from various plant sources (including maize, rice, tomato and pepper) are applicable for use in the methods of the present invention. The data from these experiments (attached as Annex A) indicates the carotenoid content of transgenic rice endosperm containing expression cassettes comprising different plant derived phytoene synthase genes. Thus it is clear that there are phytoene synthase genes described in the application as filed which are suitable for use in the methods of the invention to produce the plants of the invention and thus the amended claims are enabled.

III. Rejections Under 35 U.S.C. 103

The Examiner has cited Burkhardt et al (1996) Rice Genetics III (hereinafter D1), Bramley et al (1997) Pure and Appl. Chem. Vol 69. No.10. pp2159-2162 (hereinafter D2) and Bartley et al (1999) Eur. J. Biochem. Vol 259. pp396-403. (hereinafter D3).

D2 describes the production of a bacterial phytoene desaturase containing construct and its insertion into tomato. The results indicated that the transgenic tomato fruit contained an increased amount of carotenoids and that levels of the phytoene desaturase protein in the transgenic fruit were higher than that in the control fruit.

D2 also describes the production of a tomato phytoene synthase containing construct and its insertion into tomato. The results of this experiment however, provided a range of phenotypes and it was suggested that the 40% of transformants that contained low carotenoid levels were due to gene silencing of the phytoene synthase gene. D2 also suggests that premature deposition of lycopene (a carotenoid) limits its accumulation during ripening.

Thus D2 describes the production of two sets of transgenic plants. One containing the tomato phytoene synthase gene, which provided a variety of results and another containing the

bacterial phytoene desaturase gene. D2 essentially describes that modification of a pre-existing carotenoid producing pathway via the insertion of additional carotenoid synthesising genes is not without problems. The person skilled in the art will, upon considering D2, recognise that such modification of existing carotenoid metabolism pathway can yield quite varied results in that the carotenoid genes can be silenced, or over-expressed. There is no disclosure or suggestion in D2 that such carotenoid genes can be utilised in the production of carotenoids in plant cells that are normally carotenoid-free. This is an entirely different concept, is unconnected and simply not envisaged in D2.

With respect to D1, the Examiner has already indicated that this document does not describe a plant transformed with a bacterial phytoene desaturase encoding sequence. D1 does state that following the transformation of rice with the (a) daffodil phytoene synthase, (b) daffodil phytoene desaturase or both (a) and (b) several lines accumulated high levels of phytoene in the endosperm of mature seed but the accumulation of ζ-carotene, being the product of phytoene desaturase, was not demonstrated. There is no explanation in D1 about why ζ-carotene could not be detected.

The Examiner suggests that substitution of the phytoene desaturase of D1 with that of D2 would have been obvious. As stated above, D2 describes the modification of an already existing carotenoid production pathway and there is no indication that the pathway can be re-created in a cell that is normally carotenoid-free. In addition to this, there is nothing in D1 that would suggest an alternative phytoene desaturase is required. As previously stated, the person skilled in the art will recognise that it is no simple task to recreate a functional biochemical pathway in a cell which is normally absent the pathway. With the limited teachings of the prior art, it is only with the benefit of the certainty provided by the present invention that such an objection can be made and therefore we respectfully submit that this constitutes a "hindsight" analysis of the prior art.

D3 describes the expression of particular carotenoid biosynthesis enzymes in *Escherichia coli*. There is no description or suggestion of the expression cassettes and their use in the methods of the invention.

In summary, despite the uncertainty and limited descriptions of the prior art D1, D2, and D3, the Applicants have demonstrated that it is possible to produce plant cells that can accumulate carotenoids, which cells are normally carotenoid-free, via the methods as presently claimed. More specifically, there is no teaching in D1, D2 or D3 that would motivate the person skilled in the art to perform the method as claimed or provide a plant cell obtainable by such a method. In particular, there is no suggestion that such a polynucleotide encoding a plant phytoene synthase and a bacterial phytoene desaturase would be sufficient to allow for the production of carotenoids in cells (such as rice endosperm cells) that are normally carotenoid-free.

Annex A

			Carotenoid composition,% of total in T_1 (T_2[a]) seed				
***Psy* Source**	**Event Identity (Number of T_1 transgenic plants analysed)**	**Total carotenoid content in T_1 (T_2[a]) seed (μg g^{-1} dry weight)**	**β-carotene**	**α-carotene**	**β-cryptoxanthin**	**zeaxanthin**	**lutein**
Maize	11059-5 (6)	14.5 (14.4)	89.0 (83.3)	9.7 (10.4)	0.6 (2.6)	0.3 (1.9)	0.4 (1.7)
	11059-11 (6)	9.8 (14.2)	85.8 (84.7)	10.4 (9.5)	1.7 (2.9)	1.0 (1.6)	1.0 (1.3)
	11059-14 (5)	13.7 (16.0)	87.1 (86.0)	11.0 (9.3)	1.2 (2.3)	0.3 (1.3)	0.4 (1.1)
	11059-16 (6)	10.1 (11.8)	85.6 (85.8)	10.5 (8.9)	1.7 (2.7)	1.2 (1.5)	1.0 (1.1)
	11059-17 (6)	11.5 (16.5)	86.7 (85.0)	10.4 (9.1)	1.5 (2.6)	0.9 (1.8)	0.5 (1.5)
Pepper	7651-3 (5)	2.9 (2.1)	80.5 (72.7)	9.8 (11.2)	2.7 (4.9)	3.6 (4.9)	3.5 (6.2)
	7651-19 (5)	4.7 (5.2)	77.9 (76.6)	12.4 (11.9)	2.6 (4.5)	4.0 (4.9)	3.1 (2.1)
	7651-21 (5)	4.2 (4.9)	77.8 (78.8)	12.6 (9.9)	2.6 (5.0)	4.1 (4.0)	2.8 (2.2)
Tomato	7650-4 (5)	1.1 (2.2)	64.3 (65.9)	15.5 (9.9)	3.7 (4.7)	5.1 (9.0)	11.4 (10.6)
	7650-8 (4)	0.9 (1.3)	61.5 (58.9)	15.7 (9.8)	4.8 (6.8)	5.6 (12.3)	12.4 (12.2)
	7650-11 (2)	1.2 (2.0)	68.0 (68.4)	13.8 (11.9)	4.9 (6.7)	4.7 (6.8)	8.7 (6.2)
Rice	11586-1	13.1	81.2	13.6	1.7	1.2	2.2
	11586-12	18.4	85.0	12.2	1.0	0.7	1.0
	11586-14	11.6	86.4	9.6	1.9	1.0	1.0
	11586-20	12.5	78.4	16.1	2.2	1.3	1.9
	11586-28	8.8	84.4	10.2	2.5	1.3	1.5
Daffodil	7609-10	1.2	68.5	11.6	6.2	6.8	7.0
	7609-16	0.8	58.5	10.8	4.6	9.4	15.0
	7609-21	0.8	65.8	10.5	4.7	7.8	10.4

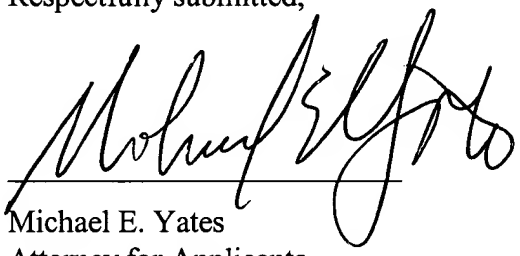
Table 2. Carotenoid content and composition of transgenic rice endosperm

[a] The number given represents the average carotenoid content of the homozygous T_2 grain analysed.

Conclusion

Applicants respectfully request that the instant amendment be entered, the above remarks be considered, and that examination of the present case proceed to allowance. The Examiner is invited to telephone the undersigned attorney at 919-541-8587 if any questions or concerns arise during examination.

Respectfully submitted,



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